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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF VASICINE AND VASICINONE IN *ADHATODA VASICA* NEES

KEITH R. BRAIN*
Welsh School of Pharmacy, UWIST, Cardiff CF1 3NU (Great Britain)
and
BHUPENDRA B. THAPA
Royal Drug Research Laboratories, Department of Medicinal Plants, Kathmandu (Nepal)
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SUMMARY

A high-performance liquid chromatographic method for the determination of the quinazoline alkaloids vasicine and vasicinone in *Adhatoda vasica* Nees and studies on the stability of vasicine in solution and in plant extracts are reported. Vasicinone appears to be an artefact of extraction and storage.

INTRODUCTION

Adhatoda vasica Nees is an evergreen bushy shrub which is widely distributed in the Indian subcontinent and has long been used in traditional medicine, in particular as a cough remedy[1,2]. The Indian Pharmacopeia[3] contains monographs on the leaf, liquid extract and syrup. *A. vasica* contains quinazoline alkaloids[4], the major component being vasicine[2,5] (Fig. 1) although vasicinone[6], which is formed by oxidation of vasicine at position 8, is also important. Vasicine is identical with peganine[7] (from *Peganum harmala*) and linarine[8] (from *Linaria* sp.). Detailed studies on the pharmacology of vasicine and vasicinone have been published, but these are somewhat contradictory. Two reports[9,10] have indicated that vasicine possesses appreciable hypotensive and bronchodilatory, and marked respiratory stimulant activities, whereas vasicinone shows relaxation of tracheal muscle *in vitro* and bronchoconstriction *in vitro*, and that a combination of equal parts of the two alkaloids shows more bronchodilatory activity both *in vivo* and *in vitro*. On the other hand

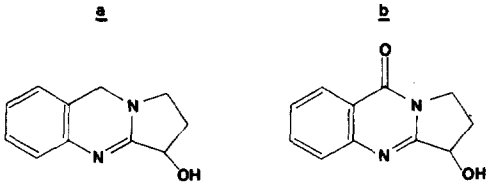

Fig. 1. Structures of vasicine (a) and vasicinone (b).

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several other workers have reported bronchoconstriction by vasicine and bronchodilation by vasicinone[11–14].

No effective quality control method, which assesses the content of individual alkaloids, has been reported for *A. vasica*. The Indian Pharmacopeia standardises on water-soluble extractive and the only other quantitative method published involves titration of the alkaloids[15]. Since vasicine is readily oxidised to vasicinone and the pharmacological effects of these compounds are different, assessment of the individual proportions of these in a material is essential. The following high-performance liquid chromatographic (HPLC) method for the rapid analysis of the individual alkaloids was therefore developed.

EXPERIMENTAL AND RESULTS

Leaves of *A. vasica* were collected from the Swayambhu forests in Nepal. On macroscopical and microscopical examination they were found to comply with the monograph in the Indian Pharmacopeia.

Isolation and identification of reference vasicine

Total alkaloid was extracted by the method of Johne *et al.*[4]. Coarsely powdered leaf (250 g) was moistened with 10% ammonia, extracted with 3 × 500 ml chloroform, and the volume reduced under vacuum to 250 ml. The chloroform layer was extracted with 10% acetic acid until free of alkaloid (Mayers reagent); then the acetic acid extract was made alkaline with 20% ammonia and extracted with 4 × 50 ml chloroform. The chloroform extract was dried over anhydrous sodium sulphate, filtered and the solvent removed under reduced pressure. Yield of crude alkaloid was 1.39%.

The crude alkaloids was dissolved in warm 90% ethanol, filtered whilst hot, and allowed to crystallise at room temperature, then recrystallised from absolute ethanol. UV, IR and mass spectra were identical with those published for vasicine[16,17].

Preparation and identification of reference vasicinone

Vasicinone was prepared from vasicine by the method of Mehta *et al.*[18]. Vasicine (200 mg) was dissolved in a mixture of 1 ml 30% hydrogen peroxide and 1.2 ml acetone, warmed to 50°C for 15 min then kept at room temperature overnight. The product was recrystallised from 95% ethanol. UV and IR spectra were identical with those published for vasicinone[18]. The mass spectrum was compared with that of vasicine and other quinazoline alkaloids and found to be consistent with vasicinone[19–23]. The ion *m/e* 146 formed from vasicinone gave three main fragments, *m/e* 130, *m/e* 119 and *m/e* 118 with the loss of H_2O, HCN and CO, respectively. The ion *m/e* 130 lost HCN to give *m/e* 103 and loss of HCN from *m/e* 118 gave *m/e* 90.

HPLC

Satisfactory separation of vasicine and vasicinone was achieved on a 30 cm × 5 mm I.D. octadecyl-silica (APEX-ODS), 5 μm, column (Jones Chromatography, Llanbradach, Great Britain) eluted with methanol (HPLC grade; Rathburn, Peebleshire, Great Britain)–dichloromethane (HPLC grade; BDH, Poole, Great Britain)–

perchloric acid (BDH) (50:50:0.01) at 1 ml/min (Fig. 3a) using a 6000M solvent delivery system (Waters Assoc., Milford, MA, U.S.A.), 7125 valve with 20-μl loop (Rheodyne, Berkeley, CA, U.S.A.) and CE 272 UV Spectrophotometer (Cecil Instruments, Cambridge, Great Britain) at 300 nm, and PM8251 recorder (Philips, The Netherlands).

Minimum detectable levels were 20 ng for vasicine and 10 ng for vasicinone and the response was linear to 10 μg for vasicine and 2 μg for vasicinone.

Degradation of vasicine

Conversion of vasicine to vasicinone has been reported[18] in solutions shaken in daylight in chloroform or benzene, during counter-current distribution in acetic acid–chloroform (10:90), and during partition chromatography on Hyflo Supercell containing 10% phosphoric acid eluted with moist chloroform. An examination of the stability of vasicine in both pure solutions and plant extracts was therefore undertaken.

Solutions of pure vasicine (about 0.3 mg/ml, accurately weighed) were prepared in 40% ethanol, methanol, ethyl acetate, chloroform, dichloromethane and toluene. In addition, to determine whether other components of a crude extract influenced the stability of vasicine, 2.5-g samples of leaf were macerated with 100 ml of each solvent, with occasional shaking, for 2 h before decantation and filtration of the extract. In the case of ethyl acetate, chloroform, dichloromethane and toluene the plant materials were moistened with 10% ammonia before extraction.

All solutions were kept at room temperature under normal laboratory lighting conditions and subjected to HPLC twice a day for 48 h. They were then subjected to continuous UV irradiation at 365 nm under a Camag UV chromatography lamp and analysed after a further 1.5 and 3 h. Results are given in Tables I and II.

From these results it was clear that the stability of vasicine varied widely according to the solvent used and whether other material was present. Pure solutions in 40% ethanol and methanol showed minimal loss, even after UV irradiation, but there were substantial losses in toluene and ethyl acetate, with the results for chloroform and dichloromethane falling between. In the case of plant extracts the initial extraction efficiency in this simple single-stage maceration process varied widely,

TABLE I

DEGRADATION OF VASICINE IN PURE SOLUTION

Solvent	*Amount added (μg)*	*Vasicine content (% of initial)*						
		In daylight (h)					*In UV (h)*	
		0	*6*	*24*	*30*	*48*	*1.5*	*3*
40% Ethanol	324	98.8	98.8	99.4	99.4	99.4	98.8	98.8
Methanol	254	99.2	95.3	97.2	94.5	97.2	99.2	97.2
Ethyl acetate	308	99.0	96.4	97.4	92.5	90.9	89.9	80.2
Chloroform	328	97.6	96.0	96.6	96.0	95.1	94.5	93.0
Dichloromethane	285	93.7	93.7	93.7	93.0	94.7	91.2	89.5
Toluene	261	100.4	100.4	97.7	96.6	95.8	92.7	78.5

TABLE II

DEGRADATION OF VASICINE IN PLANT EXTRACTS

Solvent	*Initial vasicine content (μg)*	*Vasicine content (% of initial)*					
		In daylight (h)				*In UV (h)*	
		6	*24*	*30*	*48*	*1.5*	*3*
40% Ethanol	210	103.3	100.0	97.6	100.0	98.6	97.6
Methanol	132	102.3	98.5	96.2	96.2	98.5	96.2
Ethyl acetate	265	101.9	98.1	97.0	90.6	37.7	6.4
Chloroform	285	98.9	93.0	89.5	86.7	81.4	76.1
Dichloromethane	317	100.0	98.4	96.2	83.6	83.6	75.7
Toluene	327	99.4	102.4	100.0	90.8	56.6	35.8

vasicine yield varying from 327 μg with toluene to only 132 μg with methanol. Losses by 48 h tended to be slightly higher than with pure solutions, and the effect of UV irradiation on the ethyl acetate extract, in particular, was dramatic (Fig. 2). In the ethyl acetate extract of the leaf only 6% of the vasicine remained after UV irradiation for 3 h. In contrast almost 99% of the initial vasicine content was recovered from 40% ethanol solutions under the same conditions. It is perhaps fortunate that 40% ethanol is the solvent for the standard liquid extract of *A. vasica*.

Extraction method for leaf samples

A relatively short and simple routine extraction method was desirable, with minimal possibility of degradation during extraction. Quadruple maceration with 40% ethanol effectively extracted the alkaloids but there was some interference with the vasicinone peak (Fig. 3b). This problem could be overcome by a basification and back-extraction stage (Fig. 3c). Coarsely powdered leaf (1.5 g) was extracted with 4 × 10 ml 40% ethanol, the volume reduced under vacuum to 25 ml, made alkaline

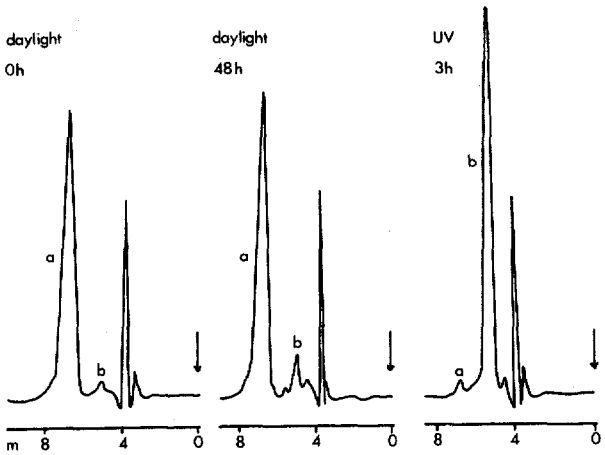

Fig. 2. Degradation of vasicine in ethyl acetate extracts of *A. vasica*. a = Vasicine; b = vasicinone. 30 × 5 cm I.D. APEX-ODS, 5 μm, column eluted with methanol–dichloromethane–perchloric acid (50:50:0.01) at 1 ml/min and detection at 300 nm.

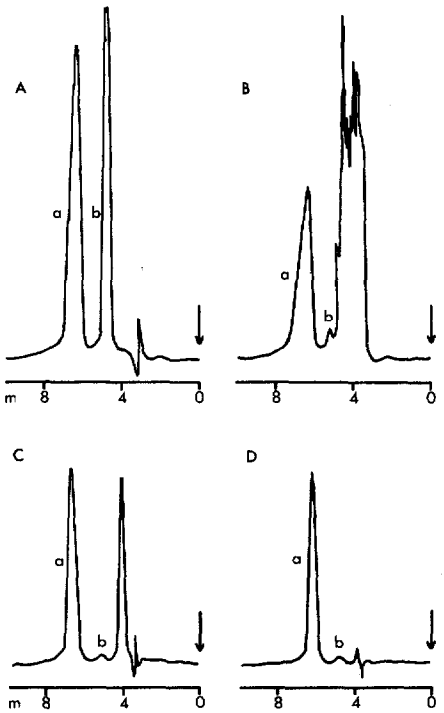

Fig. 3. HPLC of standard vasicine (a) and vasicinone (b) and leaf extracts. (A) Standards; (B) 40% ethanol extract; (C) method of Johne *et al.*[4]; (D) 40% ethanol extract after basification and back-extraction. Conditions as in Fig. 2.

with ammonia, and extracted with 4 × 10 ml chloroform. The volume was adjusted to 50 ml with chloroform, 10 ml of this diluted to 25 ml with chloroform and 20-μl samples were injected. No apparent degradation was observed during the short contact with chloroform, and this simple procedure was as effective as the full method of Johne *et al.*[4] used for extraction of reference vasicine (Fig. 3d).

TABLE III

RECOVERY OF VASICINE ADDED TO LEAF SAMPLES

Sample number	*Vasicine content of leaf (%)*	*Vasicine added (mg)*	*Vasicine found (mg)*	*Vasicine recovery (%)*
1	1.354	13.403	12.743	95.1
	1.344	13.403	13.992	104.4
2	1.500	15.125	14.932	98.1
	1.480	15.125	15.245	100.8
3	1.333	15.643	16.492	105.4
	1.331	15.643	16.392	104.8
4	1.437	10.895	10.862	99.7
	1.443	10.895	10.272	94.3
Mean	1.403			100.3

Recovery experiments

About 15 mg of vasicine, accurately weighed, was admixed with each of 4 × 1.5-g powdered leaf samples and extracted as above. Table III demonstrates the reproducibility of the method. The vasicinone content of the leaf samples was too low (less than 0.02%) for accurate estimation.

CONCLUSIONS

An effective HPLC method for the determination of vasicine and vasicinone in *A. vasica* has been developed. Degradation of vasicine is clearly influenced by solvent and UV light, but this is not a significant factor in the extraction procedure described. The very low levels of vasicinone found in fresh 40% ethanol extracts of leaf suggest that this is probably an artefact of extraction and storage. Further long-term stability trials on vasicine and plant extracts are in progress.

REFERENCES

1 D. Hooper, *Pharm. J.*, 18 (1888) 841–842.
2 *Dr. K. M. Nadkarni's Indian Materia Medica*, Vol. 1, Popular Prakashan, Bombay, 2nd ed., 1966, pp. 40–43.
3 *Indian Pharmacopeia*, Ministry of Health, Government of India, 2nd ed., 1966, pp. 792–794.
4 S. Johne, D. Groger and M. Hesse, *Helv. Chim. Acta*, (1971) 826–834.
5 J. N. Sen and T. P. Ghose, *Quart. J. Indian Chem. Soc.*, 1 (1925) 315–320.
6 A. H. Amin and D. R. Mehta, *Indian Pat.*, 62349 (1959).
7 E. Spath and E. Nikawitz, *Chem. Ber.*, 67B (1934) 45–55.
8 J. J. Willaman and Hui-Lin-Li, *Lloydia Suppl.*, (1970) 33.
9 O. P. Gupta, M. L. Sharma, B. J. Ray Ghatak and C. K. Atal, *Indian J. Med. Res.*, 66 (1977) 680–691.
10 R. N. Chopra and S. Ghosh, *Indian Med. Gaz.*, 60 (1925) 354–355.
11 A. H. Amin and D. R. Mehta, *Nature (London)*, 184 (1959) 1317.
12 M. B. Bhide, P. Y. Naik and R. B. Ghooi, *Bull. Haffkine Inst.*, 4 (1976) 43–50.
13 M. B. Bhide, P. Y. Naik, R. B. Ghooi, S. S. Mahajani and R. S. Joshi, *Bull. Haffkine Inst.*, 2 (1974) 6–11.
14 G. W. Cambridge, D. A. Jarman and A. B. A. Jansen, *Nature (London)*, 196 (1962) 1217.
15 M. Ikram and M. E. Huq, *Pakistan J. Sci. Res.*, 18 (1966) 109–110.
16 J. Holubek, O. Strouf, *Spectral Data and Physical Constants of Alkaloids*, Vol. II, Heyden, London, 1966.
17 A. K. Bhatnagar, S. S. Popli, *Indian J. Chem.*, 4 (1966) 291–292.
18 D. R. Mehta, J. S. Naravane, R. M. Desai, *J. Org. Chem.*, 28 (1963) 445–448.
19 R. S. Varma, S. P. Singh, *Indian J. Chem.*, 15B (1977) 623–624.
20 M. Z. Kirmani, S. R. Ahmed, *Indian J. Chem.*, 16B (1978) 526–527.
21 T. J. Batterham, A. C. K. Triffett, J. A. Wunderlich, *J. Chem. Soc., B*, (1967) 892–897.
22 S. C. Pakrashi, J. Bhattacharya, L. F. Johnson, H. Budzikiewicz, *Tetrahedron*, 19 (1963) 1011–1026.
23 M. K. Choudury, *Naturwissenschaften*, 66 (1979) 205.